

AWARD NUMBER: **W81XWH-16-1-0166**

TITLE: **Therapeutic Sleep for Traumatic Brain Injury**

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14. ABSTRACT This proposal will test the hypothesis that correcting sleep disorders can have a therapeutic effect on Traumatic Brain Injury (TBI). The majority of TBI patients develop sleep disorders, a correlation that is extremely prevalent in military personnel. Here, we have developed a paradigm to induce TBI in <i>Drosophila</i> . TBI induction results in increased mortality, impaired climbing behavior, decreased/fragmented sleep and cell death. We also discovered a biphasic response in both impaired climbing behavior and altered gene expression in glia cells. We are currently testing whether sleep induction can restore these impairments.					
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INTRODUCTION:

To study how TBI causes sleep disorders, and to study whether restoring normal sleep patterns, post-injury, can have a therapeutic effect, we need a model organism where baseline sleep can be recorded accurately, and where tools exist to induce either sleep or wake in such a way that TBI-induced sleep disorders can be corrected, by promoting wake in hypersomniacs and by promoting sleep in insomniacs. This approach is only feasible in *Drosophila*, as this model organism comes with a wealth of genetic tools that have been proven useful to modulate sleep and wake with high precision. We have developed a paradigm to reliably induce TBI in *Drosophila*.

ACCOMPLISHMENTS:

Major goals

We have completed most Major Tasks for Aim 1, as outlined in the Statement of Work.

Task 1A) Develop and test single fly TBI setup – **COMPLETED on 07/01/2016**

Task 1B) Determine TBI-induced sleep changes – **COMPLETED on 11/01/2016**

Task 1C) Determine TBI-induced behavioral changes – **COMPLETED on 01/01/2017**

Task 1D) Determine TBI-induced cell death – **25% completed**

Task 2A) Test the effect of sleep intervention on subsequent behavior and sleep architecture – **IN PROGRESS**

Task 2B) Test the effect of sleep intervention on subsequent markers of cell death – **ON HOLD** until Task 1D is completed

Keywords: TBI, Traumatic Brain Injury, Sleep, Gene Expression, *Drosophila*

What was accomplished under these goals?

Major Activity 1: to test the hypothesis that TBI causes either hypersomnia or insomnia in individual flies

Specific Objective 1A) Develop and test single fly TBI setup:

We have developed a *Drosophila* TBI paradigm. In this paradigm, individual flies are immobilized in a pipette tip and TBI is induced in a highly replicable manner using a solenoid. (Fig 1A). This paradigm is an improvement on previously published methods, where TBI is induced in groups of flies, using a vortexer or mechanical injury through a spring-driven contraption that. The drawbacks of these published methods are that 1) a wide variety of other injuries are induced as well as TBI and 2) the degree and severity of TBI is highly variable. By immobilizing individual flies in a 200µl pipette tip, each fly can be positioned in a highly replicable manner in front of a solenoid, a spring-driven metal pin. The solenoid itself allows precise control of the amount of force that is used to induce TBI. TBI induction resulted in reduced lifespan, where 50% of the TBI-induced flies had died 13 days after TBI induction, compared to 35 days for sham treated controls (Fig 1B).

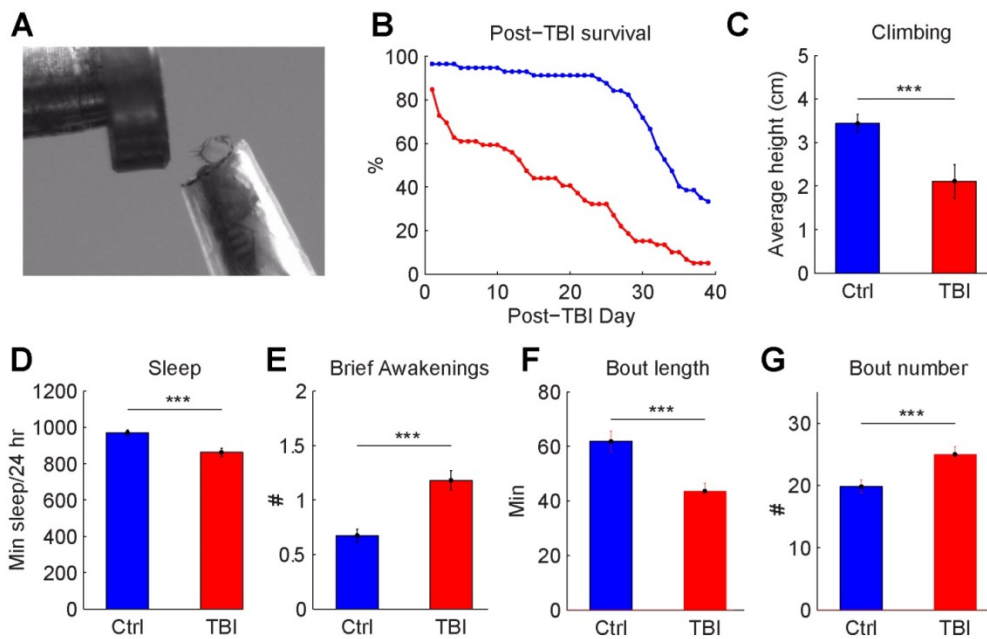


Figure 1 – TBI reduces lifespan and induces changes in climbing behavior and sleep architecture

Specific Objective 1B) Determine TBI-induced sleep changes:

To test whether TBI results in sleep changes (either insomnia or hypersomnia), individual flies were loaded into *Drosophila* Activity Monitors to quantify sleep and activity after TBI induction and compared to sham-treated controls. These activity monitors use a beam-break system to measure how active each individual fly is. Overall, TBI results in decreased sleep (Fig 1D) that is less deep (more brief awakenings, Fig 1E) and more fragmented (shorter bouts, more bouts; Fig 1F, G).

Specific Objective 1C) Determine TBI-induced behavioral changes:

To test whether TBI induction alters motor control, we performed a climbing assay. 24 hours after TBI induction, we measured how high individual flies can climb in 4 seconds. TBI induction results in a considerable decrease in climbing ability (Fig 1C).

Other Achievements: Surprisingly, the climbing defect after TBI is followed by a recovery period on post-TBI days 2 and 3, after which the climbing deficit returns on post-TBI days 4-7 (Fig 2A). To further explore the *Drosophila* immune response after TBI, we performed Translating Ribosome Affinity Purification and Sequencing (TRAP-Seq), which allows for a tissue or cell-type -specific manner of looking at the transcriptome of a given organism. This has previously been used to look at translated mRNAs in *Drosophila*. Here, we look at the glial transcriptome - glial activation is a biomarker for TBI. Following the biphasic post-TBI deficit in climbing performance (Fig 1C), we performed TRAP-seq at 1, 3 and 7 days after TBI. TBI results in a large increase in genes that are up- or downregulated on day 1 (Fig 2B). On day 3 post-TBI, the number of upregulated genes is much lower while on day 7, the number of genes that are up- and down-regulated is increased again. Contrary to our experimental design we noted that we also observed significant expression of neuronal genes suggesting that our purification is not complete. Nonetheless, our work suggests that the brain transcriptome displays a similar biphasic response to TBI. There is little overlap between the genes that are up/down regulated on days 1 and 7 (data not shown), suggesting that different mechanisms underlie this altered transcription, possibly an acute and a chronic response. Genes associated with the defense response are upregulated 24 hours after TBI, suggesting that TBI induces the activation of immune-related genes in glia after injury. Seven days after injury, genes associated with cell development, cell motion and cell morphogenesis are upregulated, suggesting that glial development and proliferation occurs after injury. Together, this biphasic response of increased expression in immune-related genes, followed seven days later by increased expression in genes associated with cell development and morphogenesis serves as a validation of our head-specific TBI model.

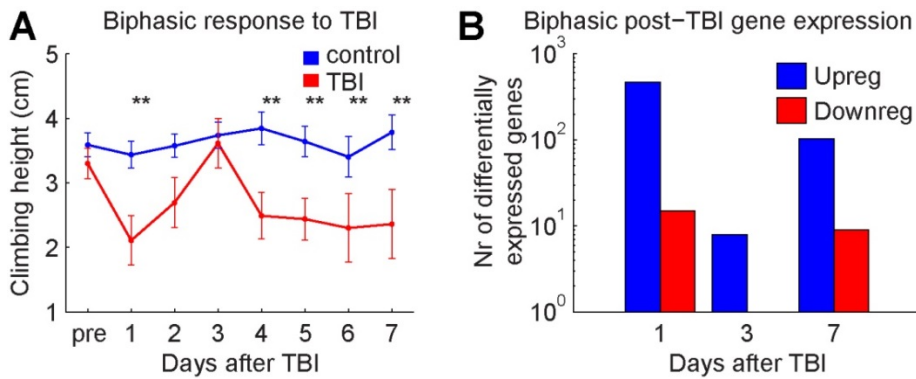


Figure 2– TBI induces biphasic changes in both climbing defects and changes in gene expression

Specific Objective 1D) Determine TBI-induced cell death

To test for cell death, a TUNEL assay was performed along with a Caspase 3 assay, for flies that were hit 1, 5 or 10 times (Fig 3). We observed TUNEL positive cells at 2 and 8 hours post-TBI in the 10 HIT but not in the 1 HIT or 5 HIT conditions. However the caspase 3 staining was negative, indicating it could be PARP-1 dependent cell death (Parthanatos) than Apoptosis. In parthanatos DNA fragmentation occurs but caspase 3 is not activated where as in necrosis neither DNA fragmentation nor caspase 3 activation happens. We are currently testing cell death at other times post TBI.

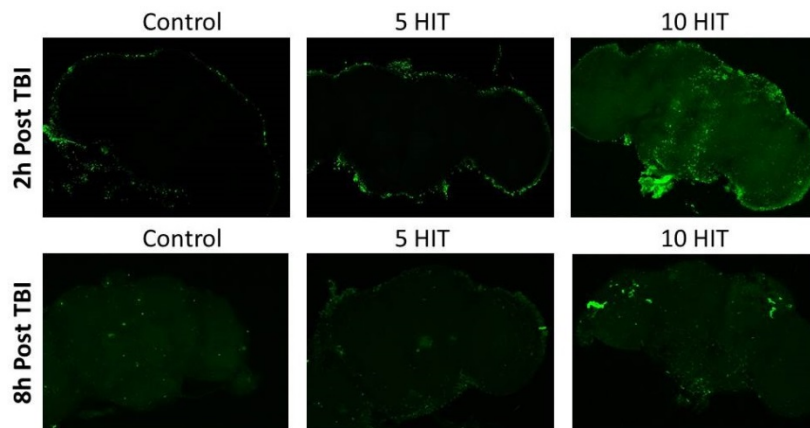


Figure 3 – TBI induces cell death

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

We have completed most of the objectives for this Major Activity. In the next reporting period, we aim to complete the quantification of TBI-induced cell death.

Major Activity 2: to test the hypothesis that correcting impaired sleep patterns can facilitate post-TBI recovery

Specific Objective 2A) Test the effect of sleep intervention on subsequent behavior and sleep architecture – IN PROGRESS

Specific Objective 2B) Test the effect of sleep intervention on subsequent markers of cell death – ON HOLD until Task 1D is completed

In this part of the project, we will test whether sleep or wake induction after TBI will alter the amount and timing of cell death. This is still on hold until we have completed Objective 1D to determine when cell death occurs after TBI.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we aim to test the effect of sleep/wake induction on post-TBI lifespan, climbing behavior and cell death.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS

Nothing to Report

PRODUCTS

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	<i>Anujaianthi Ramakrishnan</i>
Project Role:	<i>Postdoctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	<i>?</i>
Nearest person month worked:	<i>6</i>
Contribution to Project:	<i>Dr Ramakrishnan is performing the cell death assays and behavior</i>
Funding Support:	<i>-</i>

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

W81XWH-16-1-0169

6/1/16 – 11/31/17

0.9 summer, 1.5 academic

Dept. of the Army -- USAMRAA

Role: PI

Total: \$302,910

Contact: Science Officer Sarah Naylor, sarah.a.naylor.ctr@mail.mil

Title: Sleep homeostasis and synaptic plasticity

Project Goal: The goal of this proposal is to study where in the fly brain wake experience accumulates and how wake- and sleep promoting brain regions change their activity after sleep deprivation

Specific Aims:

Aim 1: to identify circuits where known modulators of sleep homeostasis modulate rebound sleep after sleep deprivation

Aim 2: To identify neural circuits where wake experience results in increased synapse formation

Aim 3: To test the hypothesis that altering synapse formation anywhere in the brain alters sleep

CBC Catalyst Award

6/1/16 - 5/31/18

0.0 academic, 0.1 summer

Chicago Biomedical Consortium

Role: PI

Total: \$250,000

Contact: Senior Associate Director Kimberly Corn, k-corn@northwestern.edu

Title: Transplanting a prokaryotic oscillator to animals to restore circadian clock function

Project Goal: The goal of this award is to transplant a bacterial clock into animals and engineer this system to drive molecular, physiological and behavioral rhythms.

Specific Aims:

Aim 1. Reconstitute function of the cyanobacterial KaiABC proteins in Drosophila tissue culture cells

Aim 2. Drive transcriptional rhythms in Drosophila cells by fusing Kai proteins to transcriptional activators

Aim 3. Using Kai-meras to drive clock gene and behavioral rhythms in flies

67885MA

10/1/2016 – 10/31/2019 0.45 academic, 0.15 summer

Dept of the Army -- Materiel Command

Role: PI

Total: \$546,186

Contact: Virginia Pasour, U.S. Army Research Office, virginia.b.pasour.civ@mail.mil

Title: Multisensory Integration by Circadian Clocks - Area 3 Mathematics (Biomathematics) and Area 8 Life Sciences (Neurophysiology)

Project Goal: The goal is to understand how circadian clocks integrate sensory information from light and temperature to entrain circadian clocks.

Specific Aims:

Aim 1. From Gene to Neuron: Integrating Transcriptomics, Optical Imaging, and RNA Interference

Aim 2. From Neuron to Circuit: Applying Connectomics and Network Modeling

Aim 3. Determining How Information from Multiple Sensory Modalities Are Integrated to Align the Clock to Environmental Cycles

Note: There is no scientific overlap between these new grants and the current proposal

What other organizations were involved as partners?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS

Not applicable